THE EFFECT OF TESTOSTERONE ALONE 
AND COMBINED WITH INSULIN 
ON THE MAMMARY GLANDS OF CASTRATED 
AND HYPOPHYSECTOMIZED RATS 

By 

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An action of testosterone on mammary gland structures was first reported by Selye et al. (1936) who found that daily injections of testosterone for 2–3 weeks caused acinar development and secretory changes in the mammary glands of normal and castrated female rats. In 1937 Astwood et al. further investigated the type of mammary gland development produced by testosterone. They showed that daily injections of testosterone developed dense clusters of alveoli in the mammary glands of normal and castrated rats. No extension of the duct tree occurred even with large doses of testosterone, but large doses given for long periods produced an abnormal mammary development with cystic formations. That testosterone stimulates mammary gland development in normal and castrated rats was subsequently confirmed by many workers (e.g. Nelson & Merckel, 1937, Noble, 1939, Reece & Mixner, 1939, Laqueur, 1942, Uyldert & Freud, 1948). However, only few of these workers have specifically described the type of mammary gland development found after testosterone treatment.

The importance of the anterior pituitary gland for the mammary gland stimulating effect of testosterone was studied by McEuen et al. (1937), Noble (1939) and Leonard & Reece (1942). These groups of workers stated that testosterone had no effect on the rat mammary gland after hypophysectomy. However, Leonard (1943) reported that in hypophysectomized rats testosterone definitely failed to induce lobule-alveolar development but that it produced a thickening of the mammary ducts. This thickening was due to hyperplasia and hypertrophy of the epithelial cells lining the ducts and to an increased diameter of the duct lumina.

Recently it has been shown that insulin is capable of making the rat mammary
gland responsive to the action of ovarian hormones in the absence of the pituitary hormones (Ahrén & Jacobsohn, 1956, Ahrén & Etienne, 1958). The question then arose as to whether treatment of hypophysectomized rats with insulin could also change the response of the mammary gland to testosterone.

In order to investigate this question it seemed necessary to obtain more information about the type and extent of mammary gland development produced in rats with intact pituitary gland by different doses of testosterone administered over various periods of time. With regard to the controversial reports mentioned above the effect of testosterone on the mammary glands of hypophysectomized rats was also reinvestigated.

In the present work the effect of various doses of testosterone propionate on the rat mammary gland has been studied in 3 groups of animals: I) castrated, female and male rats. II) castrated, hypophysectomized female and male rats, III) castrated, hypophysectomized female and male rats treated with a long-acting insulin.

**EXPERIMENTAL.**

Male and female rats from a closed colony kept at our Institute were used. The animals belonged to the same colony that had provided rats for previous work (Ahrén & Jacobsohn, 1956, Ahrén & Etienne, 1957, 1958). The food, supplied ad libitum, consisted of dog biscuits, mixed grain, bread and fresh milk. The hypophysectomized rats received glucose in the milk once a day during the period of injections.

Mammary glands of three groups of rats subjected to the following procedures were studied:

- **Group I:** Castration and injections of testosterone propionate.
- **Group II:** Castration, hypophysectomy and injections of testosterone propionate.
- **Group III:** Castration, hypophysectomy and injections of testosterone propionate combined with zinc protamine insulin (Vitrum).

In addition, some rats, only castrated or castrated and hypophysectomized, were injected with the solvent of testosterone (arachis oil). Furthermore, some of the castrated hypophysectomized rats treated with testosterone were injected with zinc protamine suspension (= control preparation to zinc protamine insulin).

The animals were castrated at 3 to 4 weeks of age. Hypophysectomy was performed by the parapharyngeal route 5 to 47 days after castration, generally 2 to 4 weeks after castration. Serial sections through the hypophysial capsule and adjacent tissues, including the pituitary stalk and median eminence, were carefully searched for remnants of the pituitary gland. Only completely hypophysectomized rats are included in the tables. A few rats with pituitary remnants are discussed under »Results».

Testosterone propionate (Neohombreol, Organon, 25 and 50 mg./ml.) was injected intramuscularly in doses of 2.5 mg. daily, 0.5 mg. every other day or daily, and 0.05 mg. daily. The volume administered daily was 0.02 ml. for all animals but for the 2.5 mg. dose given in 0.05 ml. For the dose of 0.05 mg., Neohombreol 25 mg./ml. was diluted 1:10 with arachis oil. Rats with intact pituitary gland received the first injection of testosterone 2–12 weeks after castration (see »Results«). In the hypophysectomized rats the treatment was started 19–63 days after hypophysectomy, with a majority of the experiments starting about 3 weeks after hypophysectomy.
The injections of zinc protamine insulin (40 I.U. per ml., Vitrum) were begun simultaneously with those of testosterone. Increasing doses of insulin were given subcutaneously according to the following scheme: 1 I.U. for 2 days, 2 I.U. for 4 days and 4, 6, 8, and 12 I.U. for 6 days each. Insulin was given once daily at about noon. Some rats were injected daily with a control preparation to insulin (zinc protamine suspension, Vitrum) in amounts equal to those of the insulin preparation. A 1.0 ml. tuberculin syringe was used for all the injections. In the insulin experiments the period of injections was limited by the capability of the rats to survive the treatment. The insulin treated animals were killed when they showed severe hypoglycemia, but some of them died during the night, and were then dissected early in the morning. Details about the period of the treatment are given under «Results» and in the tables.

Before the start of injections, the third right thoracic mammary gland was extirpated for whole mount preparation. This gland is referred to as the «control gland». The third left thoracic gland was taken for whole mount preparation during the period of injections or at the end of the experiment. This gland is referred to as «experimental gland». In most of the rats the injections were continued after the extirpation of the third left thoracic gland. In these rats, abdominal and/or inguinal glands were later removed and examined. These glands are also referred to as «experimental glands». In all experiments the third left thoracic gland was removed as the first experimental gland.

In most of the experiments, paraffin sections of mammary glands were studied. These sections were made from abdominal, inguinal or second thoracic glands removed in the course of the treatment (see «Results»).

The staining methods, and the histological techniques used for the whole mount preparations and the paraffin sections, as well as the criteria used in assessing the changes in the mammary glands were the same as in previous investigations (Ahrén & Etienne, 1957, 1958).

Data concerning the completely hypophysectomized rats are summarized in Tables 1–6. Changes showing in the whole mount preparations of the experimental mammary glands are graded as follows: slight but obvious changes as +, and marked changes as ++. The experimental gland was always compared with the control gland of the same rat.

The microscopic appearance of the paraffin sections is described under «Results».

The rats were weighed before each operation and at least once a week during the period of injections. At autopsy, the uterus or the prostate and seminal vesicles were examined, and, in most of the experiments, the weight of the liver and of the adrenal glands was recorded.

RESULTS

As mentioned above, different doses of testosterone propionate were used. The observations made on each of the 3 groups of rats will be described according to the following order of testosterone doses: A) 0.5 mg. every other day, B) 0.5 mg. daily, C) 2.5 mg. daily and D) 0.05 mg. daily.

Group I: Castrated rats treated with testosterone propionate.

IA: 0.5 mg. every other day.
Eleven male and 4 female rats were treated with this dose of testosterone.
Whole mount preparations of 25 mammary glands and paraffin sections of 10 glands were studied after treatment for 8–30 days. Some paraffin sections of mammary glands extirpated before the start of injections were also studied. The injections of testosterone were started 2–3 weeks after castration in 5 animals, weighing 70–90 gm. These rats had then about the same weight at the start of the injections as most of the hypophysectomized rats in groups II and III. In the remaining 10 rats the injections started 6–8 weeks after castration, when the animals weighed 160–225 gm. These 10 rats were then about the same age at the start of injections as most of the hypophysectomized rats in groups II and III.

After 8 days of treatment an effect was already clearly seen on the whole mount preparations of the experimental mammary glands. This effect consisted in a widening of the mammary ducts and a formation of alveoli grouped along these ducts (compare Figs. 1–6). With increasing length of treatment these changes were more pronounced. The alveolar development was slight to moderate in the mammary glands extirpated after 8–15 days of treatment (compare Figs. 2 and 5) and moderate to extensive after 16–30 days (compare Figs. 3 and 6). However, an alveolar development as extensive as that found in mammary glands from young adult male rats with intact testes (Ahrén & Etienne, 1957) was not seen in any gland of this group.

Paraffin sections of the mammary glands presented the following features. Before the start of injections the ducts were narrow and had a simple epithelium composed mostly of small cuboidal cells with a small amount of cytoplasm. After the treatment the ducts and alveoli were slightly dilated, and the epithelial cells were large and had an abundance of cytoplasm. In many of the smaller ducts and in most of the alveoli the epithelial cells contained large vacuoles. In most of the ducts and alveoli the epithelium was simple, but in a few of them it was composed of 2–3 layers of cells. This proliferation of epithelial cells was less extensive than that observed in glands from normal adult male rats (Ahrén & Etienne, 1957).

With the method used it was not possible to observe any major influence of the testosterone treatment on the mammary gland area.

The stimulating effect of the treatment on the mammary glands was qualitatively the same in the males and the females. Quantitatively the alveolar development was slightly more marked in the glands from the male rats. In all the female rats the nipples grew during the treatment (the males have no nipples).

The type and extent of mammary gland changes was the same in the younger rats, which received the first injection 2–3 weeks after castration, as in the older rats, which received the first injection 6–8 weeks after castration.

All the rats gained in weight during the treatment. At autopsy, the prostate and the seminal vesicles were large in the male rats, and the seminal vesicles
contained thick, yellow secretion. In the females the uteri were markedly
enlarged.

I B: 0.5 mg. daily.

This group includes 10 male and 7 female rats. Whole mount preparations
of 42 mammary glands and paraffin sections of 23 glands were studied after
treatment for 8–92 days. The time between castration and start of injections
was as follows: in 8 rats 2–3 weeks, in 7 rats 4–6 weeks, in 1 rat 8 weeks, and
in 1 rat 12 weeks.

The mammary gland changes produced by this dose of testosterone were
qualitatively similar to those described in group I A with treatment up to
30 days (Figs. 1–6 and 12–13). Quantitatively, the widening of the ducts and
the alveolar development was slightly more marked in this group. As early as
after treatment for 8–15 days many of the glands showed an extensive alveolar
development. However, the glands did not appear as thick and compact or
showed such an abundant proliferation of epithelial cells as glands from normal
young adult male rats (Ahrén & Etienne, 1957). As in the above mentioned
group the alveolar development was slightly more marked in glands from the
male rats than in glands from the females.

In some rats the injections were continued for more than 30 days, and
secretion was then more marked: the lumina of ducts and alveoli were distended
with secretion, and the epithelial cells were loaded with vacuoles (Fig. 14).
Macroscopically the secretion was colourless and did not look like milk. With
regard to the degree of distension of ducts and alveoli, there was a difference
between the glands from the males and the females. Four glands were obtained
from male rats treated for more than 50 days, and these glands showed cystic
dilatations of ducts and alveoli. These cysts were small and contained darkly
stained secretion. The epithelium lining the cysts was simple and composed of
small cuboidal or flattened cells. Seven glands of female rats treated for more
than 50 days were studied. In these glands the ducts and alveoli were only
slightly distended with secretion, and cyst formations were not seen.

The area covered by the glands did not seem to be enlarged after the
treatment. The nipples of the female rats had obviously grown. Differences
in mammary gland changes between the younger and older rats were not
found.

All the rats gained in weight during the period of injections. As in group
I A the sex accessories were enlarged in both males and females.

I C: 2.5 mg. daily.

Two male and 4 female rats were treated with this dose of testosterone.
Whole mount preparations of 17 mammary glands and paraffin sections of
15 glands were studied after injections for 10–65 days. The injections were started 6–9 weeks after castration.

The type of mammary gland changes was similar to that observed in group I A and I B, but secretion was more abundant. As early as after 14–17 days of treatment ducts and alveoli of the mammary glands from the 2 males were widely distended with secretion. After longer periods of injections the male glands showed many cyst formations containing darkly stained secretion (Fig. 15). In the glands of the 4 female rats, the secretion was less marked. The ducts and alveoli were only slightly dilated, and no cyst formations were found in spite of more than 60 days of injections (Fig. 16).

During the treatment all the rats gained in weight, and the nipples grew markedly in the females. At autopsy, the prostate and seminal vesicles were very large in the male rats, and the uteri appeared markedly enlarged in the females.

**I D: 0.05 mg. daily.**

The experiments described so far showed that treatment of castrated rats with testosterone propionate in doses of 0.5 mg. every other day to 2.5 mg. daily did not produce such thick and compact mammary glands as those found in normal young adult male rats (Ahrén & Etienne, 1957). Experiments were therefore performed in order to see whether mammary gland development similar to that observed in normal male rats would occur when a smaller dose of testosterone was administered.

Two castrated male rats, and 3 castrated incompletely hypophysectomized male rats with large pituitary remnants received daily injections of 0.05 mg. of testosterone propionate. Whole mount preparations of 11 mammary glands and paraffin sections of 8 glands from these rats were studied after treatment varying from 13 to 60 days.

After treatment for 13–22 days the experimental mammary glands presented thick ducts which began to be covered with small groups of compact alveoli. Paraffin sections showed that these ducts and alveoli were lined by 1, 2 or 3 layers of large epithelial cells (Fig. 17). Only few of the cells had vacuoles in the cytoplasm. The lumina of the ducts and alveoli were narrow. These mammary glands were very similar to those found in normal male rats at the age of 84–93 days (Ahrén & Etienne, 1957).

After treatment for about 50 days, the glands appeared very thick and compact. The ducts were covered with dense clusters of alveoli. The epithelium, lining ducts and alveoli, was composed of several layers of large epithelial cells (Fig. 18). In many parts of these glands the ducts and alveoli appeared as solid cords and clumps of large cells. In fact, these glands presented the same picture as glands from normal male rats at the age of 98–115 days (Ahrén & Etienne, 1957).
All the rats gained in weight during the treatment. At autopsy, the prostate and seminal vesicles were large but not as large as those of the above mentioned groups.

1 E: Injections with arachis oil.

Two male and 2 female rats were injected with 0.05 ml. arachis oil daily. This amount of oil is the same as that used in the present work to dissolve the highest daily dose of testosterone. Whole mount preparations of 12 mammary glands and paraffin sections of 7 glands were studied after injections for 14–60 days. The experimental mammary glands were as atrophic as the control glands.

The rats gained in weight during the treatment. At autopsy all the rats had atrophic accessory sex glands.

Group II. Castrated hypophysectomized rats treated with testosterone propionate.

Since one of the purposes of this study was to compare the results of the present group with those obtained from group III (= castrated hypophysectomized rats injected with testosterone propionate and zinc protamine insulin), some rats were injected with zinc protamine suspension (see > experimental<) together with testosterone. No differences were found between the rats injected with testosterone alone and the rats injected with testosterone + zinc protamine suspension. The rats injected with zinc protamine suspension are therefore included in this group.

II A: 0.5 mg. every other day (Table 1).

This group comprises 11 completely hypophysectomized rats. Whole mount preparations of 12 mammary glands and paraffin sections of 8 glands were studied after injections for 9 to 32 days. The mammary glands extirpated before the start of injections were atrophic and had very thin ducts (compare Fig. 7). All the experimental glands showed a thickening of the ducts (compare Fig. 8). This thickening was clearly seen in the whole mount preparations and already occurred after 9 days of treatment. In most of the rats the experimental glands seemed to have a few more side buds than the control glands. Since small but thin branches, present in the control glands, were seen more distinctly in the experimental glands because of the general thickening of the ducts, it was not possible to determine whether there was a real increase in the number of the duct branches. In some of the experimental glands the terminal ducts were markedly swollen and seemed to be composed either of clusters of small ducts or of groups of small alveoli. The glands did not present a clear lobule-alveolar development comparable with that found in group I.
Table 1.
(Group II A).
Hypophysectomized castrated rats. Injections of 0.5 mg. testosterone propionate every other day.

<table>
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1) Age at hyp. ect.: 34 days in Expt. 5; 40–47 days in Expts. 3 and 7–11; 56–67 days in Expts. 1, 2, 4 and 6.
2) exam. = examination of the experimental mammary gland.
3) castrated at the age of 5–6 weeks.

Paraffin sections revealed that the thickening of the ducts in the experimental glands was due to an increase in size and number of the epithelial cells and to a slight dilatation of the ducts (Fig. 20). The large epithelial cells had an abundance of cytoplasm. The slightly enlarged, round or ovoid nuclei, generally occupying an approximately central position in the cytoplasm of the cells, showed a loose network of chromatin and distinct nucleoli. Generally the epithelium was simple, but in a few glands it was composed of 2 layers of cells. Besides ducts of different sizes, some glands showed a few structures which appeared as groups of small alveoli or small ducts (Fig. 21). In the 3 female rats the nipples grew during the treatment.

During the injection period the body weight increased in 7 rats, remained unchanged in 1 rat and decreased slightly in the remaining 3 animals (Table 1). For all 11 rats there was an increase in body weight by an average of 7.1 %. The weight of both adrenal glands was 6.8 ± 0.51 mg. (10) or 6.0 ± 0.39 mg.

*= mean ± standard error of the mean (number of observations).
per 100 gm. body weight. The weight of the liver was 4.0 ± 0.24 gm. (10) or 3.5 ± 0.13 gm. per 100 gm. body weight. The prostate and seminal vesicles were enlarged in the males, and in the females the uterus had increased in size.

II B: 0.5 mg. daily (Table 2).

Nine completely hypophysectomized rats were injected daily with 0.5 mg.

Table 2. (Group II B).
Hypophysectomized castrated rats. Injections of 0.5 mg. testosterone propionate daily.

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1) Age at hyp. ect. 29–36 days.
of testosterone. Whole mount preparations of 21 mammary glands were studied after injections for 7–87 days. The control glands were atrophic (Fig. 7). The experimental glands extirpated after 7–12 days of treatment showed thickened ducts similar to those described for group II A. The glands extirpated after more than 2 weeks of treatment had thickened as well as dilated ducts (Figs. 8 and 9). The degree of dilatation was proportional to the length of the treatment. After 1–2 months of injections parts of the duct system were transformed into cystic formations (Fig. 9).

Paraffin sections of 17 mammary glands were studied after treatment for 9 to 87 days. After short periods of injections (9–12 days) the ducts showed an increase in size and number of the epithelial cells and also a slight dilatation as described for group II A. However, even after this short period of injections the dilatation of the ducts was more pronounced in the present group than in group II A. After longer periods of treatment, the dilatation became extensive and the epithelial cells lining the ducts were flattened. Sections of glands extirpated after 2–3 months of treatment presented large cyst formations (Fig. 22). The inner wall of these cysts was generally lined by a thin layer of flattened epithelial cells. In some of the cysts the epithelium showed a peculiar folding and papillomatous growth into the lumina.

Before the start of injections the nipples were very small in the 5 female rats. In fact, it was often difficult to detect them. As early as after 2–3 weeks of treatment the nipples were more developed. After 2–3 months of injections the nipples were extremely long (3–4 mm.) but remarkably thin. The nipples of these castrated hypophysectomized rats were longer but thinner than those of the castrated rats of group I B.

During the period of injections the body weight increased in 7 rats. In 1 rat it remained unchanged and in another one it decreased slightly (Table 2). In all 9 rats there was an increase in body weight by 13.4% (average). The weight of both adrenal glands was 6.3 ± 0.81 mg. (9) or 7.2 ± 0.56 mg. per 100 gm. body weight, and that of the liver 2.8 ± 0.27 gm. (9) or 3.3 ± 0.34 gm. per 100 gm. body weight. The accessory sex glands were markedly enlarged in both the males and the females.

Eight castrated but incompletely hypophysectomized rats were also treated with 0.5 mg. of testosterone daily. Six of these rats had large anterior pituitary remnants in contact with the infundibulum. These 6 rats were treated for up to 87 days. With regard to the body weight changes, the weight of the adrenal glands and the appearance of the mammary glands, these rats showed the same features as castrated rats with intact pituitary gland (group I B). One female rat had a few large, but not typical anterior pituitary cells in contact with the infundibulum. This rat was injected for 108 days and its body weight increased from 70 gm. to 95 gm. during treatment. The weight of the adrenal glands was 11.7 mg. (= 12.3 mg. per 100 gm. body weight). The mammary
glands studied after 13, 66 and 108 days of treatment showed the same changes as those described above for the completely hypophysectomized rats. Another female rat showed a fairly large anterior pituitary remnant encapsulated in connective tissue without detectable contact with the infundibulum. This rat was injected for 86 days, and the body weight increased from 95 gm. to 165 gm. during treatment. The mammary glands, studied after 33, 55 and 86 days of treatment, showed the same lobule-alveolar development as the glands of castrated rats with intact pituitary glands (group I B). However, the adrenal glands of this rat were atrophic (7.3 mg., or 4.4 mg. per 100 gm. body weight).

II C: 0.05 mg. daily (Table 3, Expts. 1–4).

Four completely hypophysectomized rats were treated with this dose of testosterone, and whole mount preparations of 12 mammary glands were studied

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1) Age at hyp. ect.: 49–50 days in Expt. 2, 5 and 6; 65–68 days in Expts. 1, 3 and 4.
2) Administration of zinc protamine insulin as described under «experimental».
after 12 to 90 days of treatment. The control glands were atrophic. The experimental glands showed thickened ducts, perhaps a few more side buds than the control glands, and a few markedly swollen terminal ducts. Dilatation of the ducts or cyst formations were not found. Lobule-alveolar development was absent.

Paraffin sections of 7 mammary glands showed that the thickening of the ducts was due merely to an increase in size and number of the epithelial cells (Fig. 24). In most of the ducts the epithelium was composed of more than one layer of large cells, and the lumina of the ducts appeared obstructed (Fig. 24). Slight secretion was seen in a few ducts only. Even after 50 to 90 days of treatment secretion was very scanty.

The nipples of the 3 female rats did not grow during the treatment. At autopsy, the uteri were enlarged in all three females. The size of the prostate and of the seminal vesicles of the male rat was approximately the same as that found in normal adult male rats.

II D: Injections with arachis oil (Table 4).

Four completely hypophysectomized rats were injected with 0.02 ml. arachis oil (Table 4).

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1) Age at hyp. ect.: 39 days in Expts. 4; 49–59 days in Expts. 1, 2 and 3.
2) The experimental mammary glands were as atrophie as the control glands (see »results«).
oil daily, i.e. the volume used for the injections of testosterone into the hypophysectomized rats. Whole mount preparations of 12 mammary glands and paraffin sections of 8 glands were studied after injections for 14 to 60 days. All the experimental glands were as atrophic as the control glands. The data about these experiments are given in Table 4.

**Group III. Castrated hypophysectomized rats treated with testosterone propionate and zinc protamine insulin.**

**III A: 0.5 mg. testosterone propionate every other day (Table 5).**

This group includes 11 completely hypophysectomized rats treated as group II A but, in addition, with zinc protamine insulin as described under «experimental». The mammary glands extirpated before the start of injections were atrophic. Whole mount preparations and paraffin sections of mammary glands were studied after treatment for 8–21 days. When compared with the control glands these experimental glands showed the same changes (Fig. 23) as the

**Table 5.**

(Group III A).

Hypophysectomized castrated rats. Daily injections of zinc protamine insulin, Vitrum, and injections of 0.5 mg. testosterone propionate every other day.

<table>
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<tr>
<th>Expt.</th>
<th>Body weight at:</th>
<th>Weight of both adrenals</th>
<th>Changes of the experimental mammary glands:</th>
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1) Age at hyp. ect.: 34 days in Expt. 6; 40–53 days in Expts. 2, 3, 5 and 8–11; 64–66 days in Expts. 1, 4 and 7.
experimental glands of group II A. However, the experimental glands of expts. 7 and 9 (Table 5) showed slightly more secretion than any of the glands of group II A.

All 11 rats gained markedly in weight during the period of injections. The average increase calculated for the whole group was 29.0%. The weight of both adrenal glands was $10.3 \pm 0.66$ mg. (10) or $7.3 \pm 0.71$ mg. per 100 gm. body weight, and that of the liver $7.3 \pm 0.22$ gm. (10) or $5.07 \pm 0.18$ gm. per 100 gm. body weight. The nipples grew in the female rats, and the accessory sex glands were enlarged in both the females and the males.

**III B: 0.5 mg. testosterone propionate daily (Table 6).**

This group comprises 14 completely hypophysectomized rats treated as group II B but receiving in addition zinc protamine insulin. In expts. 3 to 7 the doses of insulin were increased more rapidly than described under »experi-

**Table 6.**

(Group III B).

Hypophysectomized castrated rats. Daily injections of zinc protamine insulin, Vitrum, and injections of 0.5 mg. testosterone propionate daily.

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<th>Expt.</th>
<th>Body weight at:</th>
<th>Weight of both adrenals</th>
<th>Changes of the experimental mammary glands:</th>
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1) Age at hyp. cct.: 33–41 days in Expts. 1, 2 and 9–14; 43–52 days in Expts. 3–8.
2) The schedule of insulin was: 1, 2 and 4 I. U., each of these doses for 2 days, 6 I. U. for 3 days, and finally 8 I. U.
mental» (see Table 6). Whole mount preparations and paraffin sections of mammary glands were studied after 7–16 days of treatment. The changes observed in the whole mount preparations were similar to those found in group II B, except that the thickening of the duct system was slightly more marked in the glands of the present group. Lobule-alveolar development was not seen (Figs. 10 and 11).

As in group II B the paraffin sections of the experimental mammary glands showed an increase in size and number of the epithelial cells and signs of secretion. In addition, in the glands of the present group there was a slight increase of the connective-tissue surrounding the ducts. The fact that in the whole mount preparations, the ducts appeared slightly more thickened in the glands of this group than in those of group II B may perhaps be due to this increase of the periductal fibrous tissue.

During treatment the body weight increased in all 14 rats. The mean increase was 22.6%. The weight of both adrenal glands was 9.5 ± 0.47 mg. (14) or 8.3 ± 0.62 mg. per 100 gm. body weight, and that of the liver 6.2 ± 0.32 gm. (14) or 5.2 ± 0.25 gm. per 100 gm. body weight. The nipples grew in the female rats. In all the rats, growth of the accessory sex glands was noted.

III C: 0.05 mg. testosterone propionate daily (Table 3. Expts. 5 and 6).

Two completely hypophysectomized rats were treated with this dose of testosterone and zinc protamine insulin. Whole mount preparations and paraffin sections of experimental mammary glands showed changes similar to those described in group II D (Fig. 25). The body weight increased in both rats during treatment (Table 3), and the prostate and seminal vesicles were stimulated.

DISCUSSION

1. Experiments on castrated rats injected with testosterone.

The observations made in group I with regard to the effect of testosterone on the mammary glands of castrated female and male rats are in agreement with those of previous investigations. In the present work four different doses of testosterone propionate were used. The smallest dose (0.05 mg. daily) may be considered as physiological on the basis of the work of Moore & Price (1938) who studied the response of the prostate and seminal vesicles to various doses of testosterone in castrated male rats. This dose of testosterone propionate produced a marked thickening of the mammary duct system due to a proliferation of epithelial cells within the ducts, and a development of dense clusters of compact alveoli along the sides and at the ends of these ducts (Figs. 17 and 18). This mammary gland development is similar to that found in normal
male rats after puberty (Ahrén & Etienne, 1957). The present observations show that physiological doses of testosterone markedly stimulate the mammary glands of castrated rats. In 1950 de Graaf et al. mentioned briefly that daily injections of testosterone propionate in a dose of 40 μg. did not produce any significant development of the rat mammary gland, but since no further details are given about these experiments, a comparison between the finding of these authors and the result of the present work is not possible.

Most of the rats in group I were treated with higher doses of testosterone propionate (0.5 mg. every other day, 0.5 mg. daily or 2.5 mg. daily). With these higher doses too, a lobule-alveolar development was produced in the mammary glands, but in addition, these doses stimulated secretion (Figs. 12–16). Both ducts and alveoli were therefore more or less distended with secretion. In these distended ducts and alveoli, the proliferation of the epithelial cells was less marked than in the glands from rats treated with 0.05 mg. daily. The degree of secretion increased with the dose and the period of testosterone treatment.

Cyst formations were found in the mammary glands of male rats injected daily with 0.5 or 2.5 mg. testosterone propionate for long periods (Fig. 15). This finding is in agreement with that of Astwood et al. (1937), who found mammary cyst formations in rats injected for long periods either with high doses of androgens or with gonadotrophins. It is well known that high doses of oestrogens can also produce cyst formations in the mammary glands (for lit. see Folley, 1952). It therefore seems tempting to seek for an explanation for the fact that high doses of both androgens and oestrogens can produce a similar abnormal mammary development, whereas physiological doses of these substances produce quite different types of mammary development. Both androgens and oestrogens may act upon the anterior pituitary gland (e.g. Greep & Jones, 1950). A possible explanation might therefore be that high doses of oestrogens or androgens interfere with the secretion of the anterior pituitary hormones involved in the normal mammary gland development.

In group I, cyst formations were seen only in the glands of male rats (Fig. 15). In the mammary glands of the female rats. injected for long periods with the higher doses of testosterone, both ducts and alveoli were distended with secretion but real cyst formations were not found (Fig. 16). This observation is in agreement with that of Uyldert (1951), who found mammary cyst formations after high doses of testosterone propionate (3 mg. daily) in castrated male rats but not in castrated female rats. We have, however, incidentally observed real cyst formations in the mammary glands of a female castrated rat injected daily with 10 mg. testosterone propionate for 32 days. Differences in cyst formation in male and female mammary glands have been reported to occur after treatment with oestrogens too. Uyldert (1951) found cyst formations in the male but not in the female glands after daily injections with 100 μg. of oestradiol benzoate for 14 days. Astwood et al. (1937), on the other
hand, reported cystic changes in the female glands too after daily injections with 100–200 µg. of oestrone for 3–8 weeks. Thus, it seems that cystic formations are formed more easily in the male than in the female glands. The cause of this difference between the reaction of the male and female glands is obscure. It might be due to the fact that the mammary glands of the male rats have no nipples and therefore no outlet for the secretion, which can accumulate more readily in the male than in the female glands.

With the method used it was not possible to detect an effect of the testosterone treatment on the extension of the mammary duct system. Club-shaped end buds, characteristic of the oestrogen-stimulated mammary gland, were not developed as a result of the testosterone treatment. It therefore seems unlikely that the effect of testosterone should have been produced by oestrogens occurring during the metabolism of testosterone.

Mammary gland responses to androgens have also been studied in other species. For the mouse and the guinea-pig it has been reported that testosterone induced a slight growth of the mammary ducts but hardly any development of alveoli (for lit. see Folley, 1952). The effect of androgens on the mammary glands of the monkey has been studied by Folley et al. (1939), Van Wagenen & Folley (1939) and Speert (1948). Folley et al. injected male monkeys with testosterone, and found papillomatous outgrowths of the glandular epithelium into the duct lumina but no significant increase in the area of the glands. Alveolar development was seen in the glands of one animal treated for 151 days. Van Wagenen & Folley reported similar changes in castrated female monkeys, but testosterone did not stimulate alveolar development except in glands in which alveoli already existed. Speert treated female and male monkeys with testosterone and reported «distinct enlargement of the lobules and dilatation of the alveoli». Whether the treatment promoted alveolar development in mammary glands devoid of alveoli at the start of injections, is not clearly stated in this report. These investigations indicate that the response of the monkey mammary gland to testosterone is in many ways similar to that of the mammary glands of the rat.

II. Experiments on castrated hypophysectomized rats injected with testosterone.

The observations made in group II confirm those of Leonard (1943) who found a thickening and dilatation of the mammary ducts of hypophysectomized rats injected with testosterone propionate for a period of up to 16 days. In the present work, castrated hypophysectomized rats were treated with three different doses of testosterone propionate for periods varying from 7 to 90 days. As in group I, considerable differences were found in the mammary gland
responses to the different doses of testosterone propionate. The smallest dose (0.05 mg. daily) produced a marked thickening of the mammary duct system, which was due to a proliferation (= hypertrophy and hyperplasia) of the ductal epithelial cells (Fig. 24). The 0.05 mg. dose elicited only slight secretion which did not increase during periods of treatment of up to 2-3 months. The higher doses (0.5 mg. every other day or daily) produced proliferation of the epithelial cells as well as secretion (Figs. 19-22). After long periods of injections the secretory changes were more striking than the epithelial proliferation. After daily injections of 0.5 mg. of testosterone propionate for 2-3 months some parts of the duct system were transformed into cystic formations (Fig. 22) similar to those found in the glands of castrated rats with intact pituitary glands. To our knowledge, mammary cyst formations have not been described previously in hypophysectomized rats treated with steroid hormones.

None of the three doses of testosterone propionate produced a development of alveolar lobules, but a few terminal twigs which were more thickened than the other parts of the ducts were occasionally observed. In whole mount preparations, these swollen twigs resembled clusters of small ducts or groups of small alveoli. A differentiation of these structures could not be made with certainty from paraffin sections (Fig. 21). That it may be very difficult or even impossible to distinguish the smallest ducts from true alveoli has been pointed out by e. g. Richardson (1947). The question as to whether testosterone can stimulate limited growth of alveoli in the mammary glands of hypophysectomized rats remains therefore uncertain. However, in the present experiments it was clear that testosterone did not promote a distinct lobule-alveolar development such as that found in rats with intact pituitary glands following treatment with testosterone.

Recently Ahrén & Jacobsen (1957) observed that cortisone promoted proliferation of the ductal epithelial cells and secretion in the mammary glands of hypophysectomized rats. Experiments on parabiotic rats (Jacobsen, 1949) indicate that endogenously produced adrenocortical hormones can exert the same action on the mammary ducts as cortisone. The question then arises whether the mammary gland changes of the hypophysectomized testosterone treated rats can be explained as an effect of adrenocortical hormones. That testosterone has an effect on the adrenal glands of hypophysectomized rats was found by many investigators (for lit. see Zuckerman, 1953, Courrier et al., 1953). Testosterone injections, when started immediately after hypophysectomy, partially prevented the adrenal atrophy which results from the removal of the pituitary gland. When the treatment was started after post-operative intervals of 2-3 weeks, it had no influence on the adrenal weight but a slight stimulating effect on the cells of the adrenal cortex. In the present work, the testosterone treatment was instituted at about 3 weeks after hypophysectomy, and the adrenal glands were quite atrophic (judged from the weight) after
the treatment. It seems therefore unlikely that an increased secretion of adrenal cortical hormones plays a significant role in the mammary gland changes found in the hypophysectomized rats after testosterone treatment. This assumption is supported by the observation of Ahrén & Jacobsohn (1957) that the thickening of the mammary ducts of castrated hypophysectomized rats was not clearly recognizable until after 3 weeks of treatment with cortisone. In the present work testosterone produced a distinct thickening of the ducts as early as after 7–9 days of treatment. In addition, it may be mentioned that, in castrated rats with intact pituitary glands, cortisone given alone could stimulate secretion but did not produce alveolar development (Ahrén & Jacobsohn, 1957), whilst testosterone induced marked lobule-alveolar growth as well as secretion (Group I).

One of the incompletely hypophysectomized rats of group II B showed a fairly large anterior pituitary remnant, which was encapsulated in connective tissue and without any detectable contact with the infundibulum. During the testosterone treatment the body weight increased markedly in this rat, and the mammary gland response was similar to that found in rats with intact pituitary glands. However, at autopsy the adrenal glands were as atrophic as in the completely hypophysectomized rats. It seems therefore that the pituitary remnant of this rat was able to secrete some growth promoting substance(s) and mamnogenic hormone(s) but not ACTH. The same dissociation of the pituitary secretion has been observed in another incompletely hypophysectomized rat included in a previous investigation (Ahrén & Etienne, 1958). In connection with these observations, it may be mentioned that observations of Desclin (1956) and Everett (1956) indicate that autografts of the rat pituitary gland secrete luteotrophin but probably not ACTH.

The protein anabolic effect of testosterone in rats with intact pituitary glands is well established (Kochakian, 1950). Whether testosterone has such an effect in hypophysectomized rats seems less certain. Some investigators (Kochakian, 1950, Rupp & Paschkis, 1953) reported nitrogen retention and body weight gain. Simpson et al. (1944), on the other hand, claimed that testosterone did not cause any significant increase in the body weight of hypophysectomized female and male rats. Gordon et al. (1947) found that testosterone failed to promote a significant body weight gain in hypophysectomized rats in spite of a nitrogen retention. In the present investigation many of the hypophysectomized rats gained in weight during the testosterone treatment. In group II B this weight gain can only partly be attributed to testosterone, since these rats weighed less than 100 gm. at hypophysectomy. In our colony, rats hypophysectomized when younger than 5 to 6 weeks and weighing less than 100 gm., usually gain slightly in weight without any treatment. In group II A most of the rats weighed more than 100 gm. at hypophysectomy, and many of these rats also gained slightly in weight during the treatment. However, the small
number of control rats (group II E) does not allow of any conclusions regarding the body weight changes.

The mammary gland response to testosterone bears some resemblance to that of the sebaceous glands. Lasher et al. (1954) reported that testosterone markedly enlarged the sebaceous glands in rats with intact pituitary gland, but this effect was very limited after hypophysectomy. Ebling (1957) observed that, although the sebaceous glands were not enlarged, the cells of the glands were significantly larger in testosterone treated than in untreated hypophysectomized rats.

The effect of testosterone propionate on the mammary glands of hypophysectomized animals other than rats has hardly been investigated. Ferguson & Visscher (1953) briefly mention that no mammary gland development was seen in testosterone-treated mice after hypophysectomy.

III. Experiments on castrated hypophysectomized rats injected with testosterone and insulin.

The results obtained from group III show that the treatment with zinc protamine insulin did not change the response of the mammary gland to testosterone in castrated hypophysectomized rats (Figs. 11, 23 and 25). The only evident difference between group II and III was that some of the mammary glands of group III showed more periductal fibrous tissue. A development of alveolar lobules, such as occurs regularly in non-hypophysectomized rats injected with testosterone, was absent. Distinct growth of true alveoli has so far been obtained in hypophysectomized rats only after injections with testosterone and a growth hormone preparation (Reece & Leonard, 1942) and with ovarian hormones combined with prolactin or placental extracts (Lyons, 1951. Lyons et al., 1955). Although the action of testosterone on the mammary gland is not in all respects the same as that of progesterone, the possibility that the capacity to develop alveoli can only be restored by hypophysial or placental factors must be taken into account. Further work including studies of hypophysectomized rats treated with 1) testosterone and prolactin and 2) oestrone, progesterone and insulin in other doses than those used by Ahrén & Jacobsohn (1956) and Ahrén & Etienne (1958) seems desirable to elucidate this question.

Administration of long-acting insulin increases the food consumption and increases body weight of hypophysectomized rats (Salter et al., 1957). In the present work all the hypophysectomized rats treated with zinc protamine insulin gained in weight during the treatment. Whether the addition of testosterone was more effective than insulin alone in increasing the body weight in the hypophysectomized rats, can not be inferred from the experiments of the present work.

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Plate 1.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

5mm

(whole mounts)
COMMENTS TO PLATES I-IV

Figs. 1–11 show whole mount preparations of mammary glands stained with gallocyanin chromalum and photographed at the same magnification (see plate I). Figs. 12–25 show microphotographs of 10 µ thick sections through mammary glands stained with hematoxylin-eosin. All microphotographs are taken at the same magnification (see plate III).

Plate I.

Fig. 1: (Group I B). Castrated male rat. Age at castration 21 days. Third right thoracic gland extirpated 10 days after castration and 4 days before the start of injections. Small gland with a few side buds and end buds.

Fig. 2: Same rat as in Fig. 1. Third left thoracic gland extirpated after 10 days of treatment with 0.5 mg. testosterone propionate (T. P.) daily. Thick ducts and small groups of alveoli.

Fig. 3: Same rat as in Figs. 1 and 2. Right abdominal gland extirpated after 21 days of treatment with 0.5 mg. T. P. daily. Thick ducts and marked lobule-alveolar development.

Fig. 4: (Group I B). Castrated female rat. Age at castration 21 days. Third right thoracic gland extirpated 33 days after castration and 2 days before the start of injections. Small gland with a few side buds and end buds.

Fig. 5: Same rat as in Fig. 4. Third left thoracic gland extirpated after 10 days of treatment with 0.5 mg. T. P. daily. Thick ducts and many groups of alveoli.

Fig. 6: Same rat as in Figs. 4 and 5. Right abdominal gland extirpated after 44 days of treatment with 0.5 mg. T. P. daily. Thick ducts and extensive lobule-alveolar development.

Plate II.

Fig. 7: (Group II B, Table 2, expt. 2). Castrated hypophysectomized male rat. Third right thoracic gland extirpated 15 days after hypophysectomy and 4 days before the start of injections. Atrophic gland.

Fig. 8: Same rat as in Fig. 7. Third left thoracic gland extirpated after 21 days of treatment with 0.5 mg. T. P. daily. Thickened and slightly dilated ducts.

Fig. 9: Same rat as in Figs. 7 and 8. Right abdominal gland extirpated after 60 days of treatment with 0.5 mg. T. P. daily. Markedly dilated ducts with small cystic formations.

Fig. 10: (Group III B, Table 6, expt. 8). Castrated hypophysectomized male rat. Third right thoracic gland extirpated 28 days after hypophysectomy and 7 days before the start of injections. Atrophic gland.

Fig. 11: Same rat as in Fig. 10. Third left thoracic gland extirpated after 15 days of treatment with 0.5 mg. T. P. daily and zinc protamine insulin. Thickened ducts.

Fig. 12: (Group I B). Castrated male rat. Left abdominal gland extirpated after 21 days of treatment with 0.5 mg. T. P. daily. Ducts and alveoli slightly distended with secretion. Large epithelial cells with vacuoles in the cytoplasm.

Fig. 13: Same gland as in Fig. 12. Two large ducts with papillomatous outgrowths of the epithelium.
Plate II.

Fig. 7.  Fig. 8.  Fig. 9.

Fig. 10.  Fig. 11.

Fig. 12.  Fig. 13.
Plate III.

Fig. 14.

Fig. 15.

Fig. 16.

Fig. 17.

Fig. 18.

0.40mm (microphot.)
Plate III.

Fig. 14: (Group I B). Castrated female rat. Right inguinal gland extirpated after 56 days of treatment with 0.5 mg. T. P. daily. Ducts and alveoli with large epithelial cells loaded with vacuoles.

Fig. 15: (Group I C). Castrated male rat. Right inguinal gland extirpated after 30 days of treatment with 2.5 mg. T. P. daily. Cyst formations.

Fig. 16: (Group I C). Castrated female rat. Left inguinal gland extirpated after 60 days of treatment with 2.5 mg. T. P. daily. Ducts and alveoli distended with secretion. No cyst formations.

Fig. 17: (Group I D). Castrated male rat. Left abdominal gland extirpated after 13 days of treatment with 0.05 mg. T. P. daily. Small ducts and a group of compact alveoli. Proliferation of large epithelial cells into the lumina.

Fig. 18: Same rat as in Fig. 17. Second left thoracic gland extirpated after 50 days of treatment with 0.05 mg. T. P. daily. Ducts and compact alveoli. Proliferation of the epithelial cells. In some of the cells vacuoles in the cytoplasm. Secretion scanty.

Plate IV.

Fig. 19: (Group II A, Table 1, expt. 7). Castrated hypophysectomized male rat. Left inguinal gland extirpated 20 days after hypophysectomy and 5 days before the start of injections. Atrophic ducts with small epithelial cells.

Fig. 20: Same rat as in Fig. 19. Right inguinal gland extirpated after 21 days of treatment with 0.5 mg. T. P. every other day. Ducts with large epithelial cells slightly proliferating into the lumina.

Fig. 21: (Group II A, Table 1, expt. 6). Castrated hypophysectomized male rat. Left abdominal gland extirpated after 17 days of treatment with 0.5 mg. T. P. every other day. Group of either small ducts or alveoli such as occurring in normal males. Large epithelial cells.

Fig. 22: (Group II B, Table 2, expt. 5). Castrated hypophysectomized female rat. Right inguinal gland extirpated after 50 days of treatment with 0.5 mg. T. P. daily. Cyst formations.

Fig. 23: (Group III A, Table 5, expt. 8). Castrated hypophysectomized male rat. Left inguinal gland extirpated after 21 days of treatment with 0.5 mg. T. P. every other day and zinc protamine insulin. Ducts with large epithelial cells proliferating into the lumina.

Fig. 24: (Group II C, Table 3, expt. 2). Castrated hypophysectomized female rat. Left inguinal gland extirpated after 30 days of treatment with 0.05 mg. T. P. daily. Ducts with large epithelial cells proliferating into the lumina.

Fig. 25: (Group III C, Table 3, expt. 6). Castrated hypophysectomized male rat. Left abdominal gland extirpated after 24 days of treatment with 0.05 mg. T. P. daily and zinc protamine insulin. Ducts with large epithelial cells proliferating into the lumina.

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SUMMARY

The response of the mammary gland to testosterone alone and combined with insulin was studied in male and female rats after castration and hypophysectomy. The main results were as follows:

1) Castrated rats. Testosterone propionate promoted lobule-alveolar development and thickening or dilatation of the mammary duct system. After small doses of testosterone propionate (0.05 mg. daily), ducts and alveoli were thick and compact. After higher doses (0.5–2.5 mg. daily) the ducts and alveoli were distended with secretion. Mammary cyst formations were observed after long periods of treatment with the higher doses.

2) Castrated hypophysectomized rats. Testosterone propionate produced thickening and dilatation of the duct system but no clear alveolar development. Cystic glands were found after treatment with the higher doses. Combined treatment with testosterone propionate and zinc protamine insulin did not promote any greater mammary gland development than treatment with testosterone alone.

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